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Primed physical therapy enhances recovery of upper limb function in chronic stroke patients

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Abstract

Background: Recovery of upper limb function is important for regaining independence after stroke.

Objective: To test the effects of priming upper limb physical therapy with intermittent Theta Burst Stimulation (iTBS), a form of non-invasive brain stimulation.

Methods: Eighteen adults with first-ever chronic monohemispheric subcortical stroke participated in this randomized, controlled, triple-blinded trial. Intervention consisted of priming with real or sham iTBS to the ipsilesional primary motor cortex immediately before 45 minutes of upper limb physical therapy, daily for ten days. Changes in upper limb function (Action Research Arm Test, ARAT), upper limb impairment (Fugl-Meyer Scale, FM), and corticomotor excitability, were assessed before, during, and immediately, one month and three months after the intervention. Functional magnetic resonance images were acquired before and at one month after the intervention.

Results: Improvements in ARAT were observed after the intervention period when therapy was primed with real iTBS, but not sham, and were maintained at one month. These improvements were not apparent halfway through the intervention, indicating a dose effect. Improvements in ARAT at one month were related to balancing of corticomotor excitability and an increase in ipsilesional premotor cortex activation during paretic hand grip.

Conclusions: Two weeks of iTBS-primed therapy improves upper limb function at the chronic stage of stroke, for at least one month post-intervention, whereas therapy alone may not be sufficient to alter function. This indicates a potential role for iTBS as an adjuvant to therapy delivered at the chronic stage.

Keywords: theta burst stimulation, transcranial magnetic stimulation, rehabilitation, functional magnetic resonance imaging

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Introduction

Upper limb (UL) impairment is common after stroke and recovery of function is important for regaining independence in activities of daily living.[1] Rehabilitation of the UL involves repetitive motor practice to promote use-dependent neuroplasticity and functional recovery, and primarily occurs in the first six months after stroke.[2-4] Whether further gains are possible beyond this time has been a matter of ongoing debate.[5] Therapy may need to be primed in order to realize the potential for further gains in function at the chronic stage.[6] Non-invasive brain stimulation techniques can be used to prime the motor cortex by promoting LTP-like plasticity[7] and rendering M1 more receptive to input from other cortical areas for a greater response to therapy.[8-11]

In healthy individuals the balance of excitability between the two cerebral hemispheres is symmetric. At the chronic stage after stroke, the ipsilesional primary motor cortex (M1) is typically under-excitable and interhemispheric inhibition between the hemispheres is asymmetric, reinforcing an imbalance in corticomotor excitability between hemispheres.[12,13] Better clinical outcomes for the affected hand and arm are seen when asymmetry of corticomotor excitability is reduced.[14]

Non-invasive brain stimulation techniques that increase the excitability of the ipsilesional motor cortex may promote reorganization within ipsilesional M1 and improve the symmetry of corticomotor excitability between hemispheres.[9,10] A protocol of repetitive transcranial magnetic stimulation (rTMS), called intermittent theta burst stimulation (iTBS), may act as a priming stimulus to facilitate excitability and promote use-dependent plasticity.[15,16] Ipsilesional M1 iTBS followed by a single dose of UL practice at the chronic stage after stroke is more beneficial than UL practice alone.[17,18] However, one study has investigated the effects of multiple sessions

combining iTBS with UL therapy in chronic stroke patients, with a negative result.[19] There was no difference between real and sham treatment groups for any hand function outcome measure.

The aim of this study was to examine the effects of priming UL physical therapy with iTBS of ipsilesional M1 in subcortical stroke patients at the chronic stage. We hypothesized that UL function would be improved immediately and one month after intervention in the PRIMED Group (receiving real iTBS and physical therapy) and exceed any benefit made by the CONTROL Group (receiving sham iTBS and physical therapy). We also hypothesized improved UL function may be associated with balancing of cortical activity toward symmetry between the hemispheres, assessed with neurophysiology and neuroimaging measures.

Methods

Participants

Eighteen adults with UL impairment (Fugl-Meyer (FM) score > 20) at least 6 months after first-ever monohemispheric subcortical stroke participated in this randomized, sham-controlled, single-centred, triple-blinded trial. Volunteers were excluded if they had a brainstem or cerebellar infarction, had been diagnosed with another neurological condition,were undertaking any formal rehabilitation, or had contraindications to transcranial magnetic stimulation (TMS) or magnetic resonance imaging (MRI). They were also excluded if they were on medications that interfered with the interpretation of the neurophysiological results, or had significant aphasia or impaired cognition precluding informed consent. This study was approved by the regional ethics committee, and all participants gave written informed consent in accordance with the Declaration of Helsinki.

Design and procedures

The trial design and procedures are outlined in Figure 1. Interventions and assessments are described below. Participants were randomly allocated to PRIMED (real iTBS + physical therapy) or CONTROL (sham iTBS + physical therapy) Groups after baseline assessments were completed. Customized software (www.rando.la) was used to allocate participants and minimize between-group differences in age, baseline Action Research Arm Test (ARAT) score, the presence of a motor evoked potential (MEP) in the paretic first dorsal interosseous (FDI) muscle in response to TMS of ipsilesional M1, and fractional anisotropy (FA) asymmetry in the posterior limbs of the internal capsules.[20] Participants, assessors and the physiotherapist were blinded to group allocation. Participants were naïve to iTBS.

* Insert Figure 1 about here *

Intervention

Real or sham iTBS was delivered immediately before a 45 minute session of individualized UL physical therapy for ten consecutive weekdays. Intermittent TBS (600 stimuli[15]) was delivered to ipsilesional M1 with a biphasic Rapid Stimulator (Magstim, Dyfed, UK) by an investigator blinded to all other aspects of data collection. Sham iTBS was delivered with a sham coil (Magstim, Wales, UK). Delivery site was defined as the site on ipsilesional M1 that produced the largest MEP amplitude in paretic FDI using single-pulse TMS (i.e. the 'hot-spot'). When no MEPs could be elicited in paretic FDI, iTBS was applied to ipsilesional M1 at the mirror location of the 'hot-spot' for contralesional M1. Intermittent TBS intensity was set to 90% active motor threshold (AMT) of the nonparetic FDI. AMT was obtained as the participant was performing an isotonic contraction of the nonparetic FDI at around 20% maximum

voluntary contraction. It was defined as the minimum stimulus intensity that produced a peak-to-peak MEP amplitude >100 μV in four of eight consecutive trials, similar to established guidelines.[21] Delivery site and nonparetic AMT were determined each session. Participants remained at rest during and for 5 minutes after iTBS delivery.

UL therapy was commenced five minutes after iTBS, to allow time for consolidation before movement.[22] Therapy consisted of 45 minutes of UL exercises (strengthening, task-specific and functional tasks) delivered by an experienced neurological physiotherapist. Therapy content was individualised to each participant, based on the therapists own assessment.

Assessment

Clinical and neurophysiological assessments were completed before the intervention period on two occasions, separated by two weeks (Base1 and Base2). Assessments were repeated midway through the intervention (MID) and immediately (IMMED), one month (1M) and three months (3M) post-intervention. MRI studies were conducted two weeks before (MRI_{BASE}) and one month after (MRI_{POST}) the intervention.

The National Institutes of Health Stroke Scale (NIHSS) and the modified Rankin Scale (mRS) were used to evaluate stroke severity and disability at baseline. UL function and impairment were evaluated with the Action Research Arm Test (ARAT[23]) and the UL component of the Fugl-Meyer Scale (FM[24]) respectively. Assessors were blinded to group allocation, and not involved in treating participants.

TMS was used to evaluate corticomotor excitability. MEPs were recorded from FDI bilaterally using standard surface electromyography (EMG) techniques. Signals were amplified using Grass P511 amplifiers (Grass Instrument Division, Warwick, RI), band-pass filtered at 20 –

1000 Hz, sampled at 2 kHz and stored for subsequent analysis. Rest motor threshold (RMT) was determined for each FDI, defined as the minimum stimulus intensity that produced a peak-to-peak MEP amplitude ≥ 50 μV in four of eight consecutive trials, similar to established guidelines .[21] Stimulus-response curves were constructed by recording blocks of 12 MEPs (4 – 5 seconds between stimuli) at intensities -5%, +5%, +15%, +25% and +35% of maximum stimulator output (MSO) relative to RMT, with the order of stimulus intensities randomized. Trials were rejected online when root mean square EMG (rmsEMG) calculated over a 100 ms window prior to the stimulus exceeded 10 μV. The average MEP amplitude at each stimulus intensity was calculated, and the slope of the linear portion of the stimulus-response curve was estimated. Slope was set to zero when no MEPs could be elicited. Interhemispheric balance of corticomotor excitability was quantified by calculating an asymmetry index from the slope value (mV/10%MSO) for the contralesional and ipsilesional hemisphere: $CE_{AI} = (CE_{contra}-CE_{ipsi})/(CE_{contra}+CE_{ipsi})$. The asymmetry index was set to 1.0 when no MEPs could be elicited from the ipsilesional M1. The change in corticomotor excitability asymmetry index (ΔCE_{AI}) was calculated for participants with MEPs in the paretic FDI.

For neuroimaging, all T1-weighted and diffusion-weighted images were acquired with a Siemens 1.5 T Avanto scanner. Axial T1-weighted images were used to identify lesion location and had 1.0 x 1.0 x 1.0 mm voxels, a 256 mm field of view, TR=11 ms, and TE=4.94 ms. Diffusion-weighted images had 1.8 x 1.8 x 3.0 mm voxels, a 230 mm field of view, b=2000 $s.mm^2$, TR=6700 ms, TE=101 ms, 30 gradient directions and two averages. All image processing was carried out with the Oxford FMRIB Software Library.[25] The mean FA was calculated within the posterior limb of each internal capsule (PLIC) by warping a template PLIC volume of interest to the participants' images.[26] The structural integrity of the PLICs was

quantified by calculating an asymmetry index from the mean FA values: $FA_{AI} = (FA_{contra} - FA_{ipsi})/(FA_{contra}+FA_{ipsi})$.[20,26]

The functional MRI (FMRI) experiment was an event-related handgrip task.[27] Blood oxygen level-dependent (BOLD) contrast images were acquired using a T2*-weighted single-shot gradient echo EPI sequence (TR=3000 ms, TE=50 ms, 3 mm isotropic voxels, axial slices, 90° flip angle, 64 x 64 matrix). MRI compatible force transducers (Biopac Ltd) were placed in each hand, with the transducer in the paretic hand used to generate the visual feedback signal,[27] and the other transducer used to monitor the presence of mirror movements. Each scanning run lasted 6 min and contained 45 events separated by interstimulus intervals of 7 ± 2 s. Two scanning runs were performed with each hand for a total of 90 events per hand.

Each event consisted of paretic hand grip to a specified level of force that was displayed visually using custom software and a projection system. Force targets were varied pseudo-randomly between 10%, 15%, 25%, 30% and 35% of MVC for the paretic hand. Each target force was maintained for 1.7 s until a cue to 'relax' appeared. Rest intervals varied between 7-9 s and served as an implicit baseline.

Image processing for fMRI was performed using FMRIB's software library and FMRI expert analysis Tool, (FEAT V5.98).[28,29] Images were motion and slice-time corrected, spatially smoothed (5 mm) and then co-registered to the T_1-weighted image and spatially normalized to the Montreal Neurological Institute (MNI) template.[30] Each grip was modelled with the height (force) scaled relative to the MVC and convolved with a gamma-shaped hemodynamic response function, along with its time derivative. The six motion parameters computed by MCFLIRT were modelled as nuisance variables, as was the handgrip force signal from the good hand (not

involved in the task). FMRI statistical analyses were determined from a contrast of handgrip force computed for each scan (first-level analysis). A second-level analysis combined both scans in a fixed effects model for each participant at each time point (MRI_{Base} and MRI_{POST}). Both first and second level analyses used a corrected cluster threshold of Z>2.3 and significance threshold of p=0.05. For participants with a left hemisphere lesion, images were left-right flipped such that lesions were always on the right side to assist with group-level analyses.

In FEAT, MRIpost - base difference images were computed from 2nd level analyses, and a 2 Group unpaired t-test was conducted at a third-level (PRIMED versus CONTROL). An additional third-level analysis was conducted independent of Group to determine which brain areas (Post) were positively associated with ΔARAT and ΔFM (fixed effect, single group plus covariate de-meaned). Results of third-level analyses were masked using the HMAT template with a corrected cluster threshold of Z>2.3 and significance threshold of p=0.05. Anatomical locations of activation foci were identified probabilistically using the Juelich Histological Atlas.[31]

Supra-threshold voxels from Z-statistic images were identified within the Hand Motor Area Template,[32] which consists of bilateral primary motor (M1) and sensory (S1) cortex, dorsal and ventral premotor (PMd, PMv) cortex, and pre- and proper supplementary motor area (preSMA, SMA). A laterality index (LI) was calculated as: $LI = (NV_{contra}–NV_{ipsi})/(NV_{contra}+NV_{ipsi})$, where NV_{contra} and NV_{ipsi} are the number of suprathreshold voxels within contralesional areas and ipsilesional areas respectively.[20,33] ΔLI was calculated ($MRI_{POST} – MRI_{BASE}$) such that negative values reflect a change toward more normal activation during the task.[27]

Statistical analysis

The primary endpoint was the improvement in UL function at one month, measured with the ARAT. The secondary endpoints were UL impairment (FM), corticomotor excitability asymmetry index (CE_{AI}) and FMRI lateralization (LI) at one month. ΔARAT, ΔFM and ΔCE_{AI} were calculated at each time-point, by subtracting the baseline from the scores at MID, IMMED, 1M and 3M. For FMRI lateralization, ΔLI was calculated as LI_{POST}-LI_{BASE}.

Baseline, ΔARAT and ΔFM data were analyzed using repeated measures analysis of variance (RM ANOVA) with the between-subject factor Group (PRIMED, CONTROL), and Time and Hand (when appropriate) as the within-subject factors. Kolmogoroz-Smirnov tests were used to confirm normality. Post hoc two-tailed independent t-tests and two-tailed one-sample t-tests were used to investigate significant effects. Two-tailed independent t-tests were conducted to compare ΔLI between Groups.

Regression analyses were used to determine associations between the change in clinical measures (ΔARAT and ΔFM) and the change in corticomotor excitability asymmetry (ΔCE_{AI}) immediately and 1M after the intervention, independent of Group. Regression analyses were also used to determine associations between the change in clinical measures and the change in lateralisation of brain activation from FMRI (ΔLI) at 1M, independent of Group. For the FMRI data, a further analysis was conducted independent of Group to determine which brain areas at MRI_{POST} were positively associated with ΔARAT and ΔFM at 1M.

Baseline measures were analyzed with independent two-sided t-tests for linear continuous variables and two-tailed Pearson Chi-Square Tests for nominal and ordinal variables, except when expected cell counts were less than five, in which case two-tailed Fisher's exact tests were used. Modified Bonferroni correction was used for multiple comparisons.[34] Significance level was p=0.05.

Results

The PRIMED and CONTROL Groups were well-matched at baseline (Table 1). RMT was analyzed with an RM ANOVA, with factors Hand (Paretic, Non-paretic), Group and Time (Base1, Base2). RMT was higher in the paretic FDI (68 ± 6 %MSO) than the non-paretic FDI (40 ± 2 %MSO), as expected ($F_{1,16}$=19.2, p<0.001). For each hemisphere RMT was similar between Groups and stable across baseline (both p>0.05), and there were no interactions (all p>0.4). Contralesional FDI AMT was similar for the PRIMED (51 ± 7 %MSO) and the CONTROL Groups (49 ± 8 %MSO) (two-tailed independent t-test, p>0.5). Intermittent TBS stimulation intensity was similar for the PRIMED (46 ± 6 %MSO) and CONTROL Groups (44 ± 7 %MSO) (two-tailed independent t-test, p>0.5). Intermittent TBS protocols were well tolerated by participants, with no adverse effects. There was no mortality, seizures, hospital visits or recurrent strokes in either group.

* Insert Table 1 about here *

Upper limb function

The second baseline ARAT scores (Base2) were used to calculate ΔARAT, as there was a significant effect of Time on the two baseline scores ($F_{1,16}$=8.4, p=0.010). A small increase in mean ARAT score was seen (Base1=28.9, 95% CI 20.6 – 37.2; Base2=30.4, 95% CI 22.2 – 38.7), probably due to increasing familiarity with the testing procedure. There was no effect or interaction with Group (both p>0.3).

Upper limb function improved in the PRIMED but not CONTROL Groups immediately and at one month after intervention. There was a main effect of Group ($F_{1,16}$=14.2, p=0.002), and no

effect or interaction with Time (both p>0.2) (Figure 2A). The main effect of Group arose because ΔARAT was greater for the PRIMED than the CONTROL Group (PRIMED=1.9, 95% CI 1.1–2.8; CONTROL=-0.19, 95% CI -1.0–0.65). The increase in ARAT score was greater than zero immediately after the intervention (IMMED) for the PRIMED Group (mean ΔARAT=3.2, $t_{1,8}$=3.7, p=0.006), but not the CONTROL Group (mean ΔARAT=-0.4, $t_{1,8}$ = -1.1, p=0.312). The increase in ARAT was also greater than zero at the primary endpoint (1M after treatment concluded) for the PRIMED Group (mean ΔARAT= 2.0, $t_{1,8}$=4.0, p=0.004), but not the CONTROL Group (mean ΔARAT=0.2, $t_{1,8}$=0.4, p=0.719). In addition, the change in ARAT score was higher for the PRIMED than CONTROL Group immediately after the intervention (IMMED: $t_{1,16}$=3.8, p=0.002), and at the primary endpoint (1M: $t_{1,16}$=2.3, p=0.036). ΔARAT did not differ from baseline, nor differed between groups, at the MID or 3M time-points (all p>0.1).

* Insert Figure 2 about here *

Upper limb impairment

The two groups were matched at baseline for UL impairment, with no effect of Time or Group, and no interaction between them (all p>0.4). For consistency, FM scores at the second baseline were used to calculate ΔFM.

Upper limb impairment improved in both the PRIMED and CONTROL Groups immediately after intervention. There were no differences between the PRIMED and CONTROL groups for the change in FM score, or any interaction with Time (both p>0.7). The was an effect of Time ($F_{3,48}$=3.4, p=0.025), as FM score was greater immediately after the intervention (IMMED: $t_{1,17}$=3.4, p=0.004), but at no other time points (all p>0.07) (Figure 2B).

Regression analyses

Greater improvement in ARAT score was associated with a shift towards more balanced corticomotor excitability (negative ΔCE_{AI}) 1 month after intervention. There was a weak negative correlation between ΔCE_{AI} and ΔARAT at the 1M primary endpoint (R^2=0.269, p=0.028) (Figure 2C). There was no association between ΔCE_{AI} and ΔARAT, or ΔCE_{AI} and ΔFM, at the IMMED time-point (both R^2<0.16, p>0.8). MEPs were present in the paretic FDI in 13 participants. Slope was set to zero and CE_{AI} was set to 1.0 for the remaining five participants.

Functional MRI

At baseline, paretic hand task performance was associated with bilateral cortical activity, which was more lateralised to the ipsilesional hemisphere for less impaired participants (Figure 3).

* Insert Figure 3 about here *

At baseline, the group average activation (max Z=12.2) had peaks probabilistically located within the ipsilesional hemisphere across the corticospinal tract white matter (78%), primary motor cortex BA4a (60%) and BA4p (7%), and premotor cortex BA6 (14%) (Figure 3A). One month after the intervention the group average activation (max Z=16.1) had peaks similarly located within the ipsilesional hemisphere in the primary motor cortex BA4p (80%) and BA4a (46%), primary somatosensory cortex BA3b (34%) and corticospinal tract white matter (24%) (Figure 3B). From inspection of Figure 3(A and B), the activation spanned both hemispheres into all regions of the HMAT, with the peak of activation centered near the hand knob,[35] and M1/S1 border of the central sulcus of the ipsilesional hemisphere. Conversely in the contralesional

hemisphere, there was activation in the premotor, supplementary and secondary sensory regions, but activation was largely absent along the M1/S1 boundary and central sulcus. This indicates the contralesional activation pattern was unlikely to represent non-paretic hand involvement.

Results from two participants at baseline are shown in Figure 3C and illustrate the extent to which activation was lateralized depending on impairment level. The red-yellow map is the task-related activation from a participant with mild impairment and good function at baseline (ARAT=55; FM=54). Activation was more lateralized and contained predominantly within the ipsilesional hemisphere. The blue-light blue map is from a participant with more severe impairment and poor function (ARAT=14; FM=26). This participant had more bilateral task-related activation including cortical areas beyond those defined by the HMAT.

The change in the laterality index after the intervention did not differ between the PRIMED and CONTROL groups (ΔLI; $p=0.52$). When the groups were combined, there was no significant activation in the average pre-post and post-pre contrasts, indicating there was no consistent pattern of cortical reorganization across participants.

Changes in UL function and impairment were related to two distinct patterns of activation post-intervention (Figure 4). Improvements in ARAT were associated with activation peaks (2193 voxels, max Z=6.54) in ipsilesional premotor cortex BA6 (52%) and primary motor cortex BA4a (4%). Improvements in FM were associated with activation peaks (1857 voxels, max Z=4.9) in ipsilesional primary somatosensory cortex BA1 (50%), BA2 (25%) and BA3b (19%), primary motor cortex BA4a (13%) and BA4p (5%) and superior parietal lobule 5L (4%). Activation in these areas was associated with positive linear trends in the respective clinical scores.

* Insert Figure 4 about here *

Discussion

Two weeks of iTBS-primed physical therapy improved UL function in people who were on average 20 months post-stroke. The improvement in ARAT score was modest, however it exceeded the intra-rater limits of agreement.[36] Physical therapy alone was not sufficient to produce functional gains, possibly because the dose was inadequate. This study provides some insight into adequate doses of iTBS-primed therapy, as two weeks but not one week of treatment was required to produce improvement in UL function. Ten days of iTBS-primed therapy produced improvements that persisted for at least one month, but not three months. Longer treatment periods may produce more lasting benefits, and regular re-treatment may be needed to maintain these benefits long term.

For a lower therapy dose, priming may be required to maximise recovery potential in the chronic phase after stroke. Two weeks of high dose upper limb therapy such as modified constraint-induced movement therapy (CIMT) can produce lasting functional gains, but is only suitable for people with relatively mild impairment.[37] A more recent study found that one hour of upper limb therapy delivered daily for two weeks did not alter motor function in people only 8 months post-stroke.[38] However when iTBS was delivered on the same days as therapy, motor function and reaction time of the paretic hand improved. The timing of iTBS relative to therapy was not reported in this previous study making it difficult to identify the potential mechanism underlying these gains. The present results indicate that iTBS may specifically prime the ipsilesional hemisphere in a way that increases the effectiveness of a low dose of UL therapy.

Intermittent TBS may have created a permissive environment for cortical reorganization in response to therapy by facilitating cortical excitability, promoting LTP-like plasticity[7] and modifying the receptiveness of M1 to input from other cortical areas.[11] These effects may have

contributed to improvements in the planning, fractionation and coordination of paretic UL movement, reflected by an increase in ARAT score that persisted for at least one month. This interpretation is supported by our finding that improvement in UL function was related to rebalancing of M1 excitability, as hypothesized. Improvement in UL function was also related to an increase in ipsilesional dorsal premotor cortex activation during paretic hand grip. Dorsal premotor cortex is implicated in organising and selecting motor output, and through its dense connections with M1 is thought to make an important contribution to the recovery of motor function after stroke.[39]

Upper limb impairment was reduced immediately after the intervention, regardless of whether participants were primed with real or sham iTBS, but this improvement did not persist one month later. Therapy is likely to have had both central and peripheral effects. Centrally, the increase in FM score was related to increased ipsilesional sensorimotor cortex activation during paretic hand grip, but not rebalancing of M1 excitability. The former may reflect enhanced processing of sensory feedback during paretic hand use. Peripheral effects such as reconditioning of muscles, joints and connective tissue may also contribute to reduced impairment. Although two weeks of therapy temporarily reduced impairment, this did not translate to improved function unless therapy was primed with iTBS.

The strengths of this study are the blinding of participants, therapists and assessors, and the balancing of groups at baseline. The multimodal design provided some insight into the underlying mechanisms of the observed clinical benefits of the intervention, which was brief, non-invasive and well-tolerated. A potential limitation is that the resources required to conduct the repeated neurophysiological and neuroimaging measures constrained the study's sample size.

However, future trials may focus on clinical outcomes, rather than underlying mechanisms, enabling larger sample sizes.

In conclusion, iTBS priming of the ipsilesional hemisphere is an effective adjuvant to upper limb physical therapy for patients at the chronic stage. Larger gains in motor function at the chronic stage may be realised with primed therapy, as opposed to therapy alone. It appears that more than one week of primed therapy is required to produce improvements in motor function, and more than two weeks of primed therapy may be required to produce lasting benefits. Primed therapy promoted rebalancing of corticomotor excitability, and this was associated with greater improvements in UL function, confirming previous work with patients at the chronic stage of recovery. Improvements in UL function and impairment were associated with different patterns of cortical reorganization, and this may be a useful area of future research.

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Conflict of interest/Disclosure: None

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Table: Baseline characteristics for randomized participants.

	PRIMED	CONTROL	p value
Median age (range, years)	61 (21 – 80)	71 (38 – 79)	0.23
Sex	3 F, 6 M	3 F, 6 M	1.00
Lesioned hemisphere	3 L, 6 R	1 L, 8 R	0.58
Median months post-stroke (range)	20 (6 – 72)	18 (7 – 56)	0.58
Median NIHSS (range)	3 (0 – 4)	2 (0 – 5)	0.18
Median mRS (range)	2 (2 – 4)	2 (1 – 4)	0.78
Mean UL-FM (range)	38 (23 – 63)	40 (21 – 56)	0.88
Mean ARAT (range)	31 (13 – 51)	30 (5 – 55)	0.93
Paretic FDI MEPs	6 Y, 3 N	7 Y, 2 N	
Mean CE asymmetry (range)	0.887 (0.33 – 1.00)	0.541 (-0.16 – 1.00)	0.08
Mean FA asymmetry (range)	0.215 (0.050 – 0.571)	0.258 (-0.033 – 0.485)	0.61

Independent two-sided t-tests were used for linear continuous variables. Two-sided Pearson Chi-Square Tests were used for nominal and ordinal variables, except when expected cell counts were less than five, in which case two-sided Fisher's exact tests was used. NIHSS = National Institutes of Health Stroke Scale. mRS = modified Rankin Scale. UL-FM = Upper limb Fugl-Meyer Scale. ARAT = Action Research Arm Test. FDI = First dorsal interosseous. MEPs = Motor evoked potentials. CE = Corticomotor excitability. FA = fractional anisotropy of the posterior limb of the internal capsule.

Figure legends:

Figure 1. Trial profile and procedures.

A. Trial profile. **B**. Trial procedures. Clinical and neurophysiological assessments were completed before (Base1, Base2) and during (MID) the intervention, and immediately (IMMED), one month (1M) and three months (3M) after the intervention. Functional magnetic resonance imaging was completed at baseline (MRI$_{BASE}$) and one month after the intervention (MRI$_{POST}$). Intervention was either Real or Sham iTBS prior to 45 min upper limb therapy for ten consecutive weekdays.

Figure 2. Upper limb function and impairment.

A. Change in Action Research Arm Test score (ΔARAT) for the PRIMED (black bars) and CONTROL (light gray bars) Groups across time. There was a main effect of Group ($p=0.002$). ARAT score improved (positive ΔARAT) for the PRIMED compared to the CONTROL Group. For the PRIMED Group ΔARAT was positive immediately and one month after intervention, and was significantly greater than for the CONTROL Group (all $p<0.04$). **B**. Change in UL Fugl-Meyer score (ΔFM) across time (patterned bars). There was a main effect of Time ($p=0.025$). FM score increased (positive ΔFM) compared to baseline immediately after intervention. For both figures: MID = midway through intervention. IMMED = immediately after intervention. 1M = one month after intervention. 3M = Three months after intervention. Error bars = standard error; ^ $p<0.05$, two-tailed t-test comparison with baseline. * $p<0.05$, ** $p<0.01$, two-tailed t-test comparison between Groups. **C**. Regression analysis between ΔARAT score (positive values indicate improvement; negative values, deterioration) and Δ corticomotor excitability asymmetry

index (CE_{AI}) (negative values indicate less asymmetry) at one month after intervention (1M) for the PRIMED (black symbols) and CONTROL (light gray symbols). Data points are participants.

Figure 3. **Functional MRI results**

Event-related FMRI was performed for visuomotor force handgrip task performed with the paretic hand (n=16). **A**. Group average activation at Baseline and **B.** Primary Endpoint. Colours show range above threshold (2.3) to max=17.3. The background anatomical image is the MNI152_1mm_brain, radiological convention (right hemisphere, image left). Transparent colours over frontal and parietal cortical regions outline the 12 areas of the Hand Motor Area Template (HMAT) used for pre-threshold masking of Z-statistics at the group level. **C**. Individual FMRI results from two participants (red = mild impairment; blue = severe impairment) at Baseline (overlaid on MNI template and HMAT as above). Crosshair is located at MNI coordinate (mm 35, -22, 57) from group peak of activation at baseline (A above). Colours show range above threshold (2.3) to max=10.3.

Figure 4. Functional MRI covariate analyses.

Group-level covariate analyses post-intervention found ΔARAT (blue-light blue) was associated with increased activation in ipsilesional premotor cortex, and ΔFM (red-yellow) was associated with increased activation in ipsilesional sensorimotor cortex. Colours show range above threshold (2.3) to max=10.3.The background anatomical image is the MNI152_1mm_brain, radiological convention (right hemisphere, image left). Transparent colours over frontal and parietal cortical regions outline the 12 areas of the Hand Motor Area Template (HMAT) used for pre-threshold masking of Z-statistics at the group level.